R-Biopharm
RIDASCREEN FAST Aflatoxin SC

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GENERAL INFORMATION

The RIDASCREEN FAST Aflatoxin Single Control (SC) test method, product number R9002, is a competitive enzyme immunoassay for the quantitative analysis of aflatoxins in barley, rice, corn, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, corn starch, distillers dried grains, distillers dried grains with solubles, malted barley, milled rice, oats, popcorn, rice bran, rough rice, rye, sorghum, soybeans, wheat, wheat flour, and wheat middlings. The test kit is limited to providing quantitative aflatoxin measurements between 5 – 100 parts per billion (ppb).

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	*R-Biopharm Inc. 877-789-3033*
Test Kit Name:	RIDASCREEN FAST Aflatoxin SC
Product Number:	R9002
Effective Date of Instructions:	04/21/2016
Instructions Revision Number	0
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, barley, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, corn starch, distillers dried grains, distillers dried grains with solubles, malted barley, milled rice, oats, popcorn, rice bran, rough rice, rye, sorghum, soybeans, wheat, wheat flour, and wheat middlings.

Extraction method:	Blend 50-gram sample with 250 mL of 70% methanol/30% water (v/v) using a blender set to high speed for 2 minutes.
Test Format:	Microtiter well plate assay.
Detection Method:	Stat Fax Reader, Model 303 Plus

PREPARATION OF TESTING MATERIALS

Wash Solution:

(1) To prepare the Wash Solution, dissolve the contents of the packet containing the buffer salt in 1 liter of distilled or deionized water. Document the technician's name, preparation date, and expiration date on wash solution bottle. A removable gum-label affixed to bottle for documentation is recommended.

(2) Swirl to mix before use. When stored properly (at 36 - 46º F) the solution has a shelf life of four weeks.

(3) Alternative Preparation of Wash Solution:

(a) Dissolve the contents of the packet in only 100 mL of distilled or deionized water to obtain a 10 fold concentrated washing buffer. This solution expires after approximately 8 weeks when stored at room temperature (68 - 77º F).

(b) Use 1 part of the concentrated washing buffer and dilute with 9 parts of distilled or deionized water to obtain the ready-to-use wash solution.

Example: 100 mL of this concentrated washing buffer should be mixed with 900 mL of distilled water to get 1000 mL of wash solution.

Extraction solvent:

(4) To prepare the extraction solvent, add 2800 mL of ACS grade methanol to a clean 4L bottle. Next add 1200 mL of distilled or deionized water. Document the technician's name, preparation date, and expiration date on wash solution bottle. A removable gum-label affixed to bottle for documentation is recommended.

SAMPLE PREPARATION AND EXTRACTION PROCEDURES

Standard Extraction Procedure for corn, barley, corn bran, corn flour, corn germ, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, corn starch, distillers dried grains, distillers dried grains with solubles, malted barley, milled rice, oats, popcorn, rice bran, rough rice, rye, sorghum, soybeans, wheat, wheat flour, and wheat middlings.

(1) Weigh 50 ± 0.2 grams ground sample into a blender jar.

(2) Add 250 mL of extraction solvent [70% methanol/30% distilled or deionized water (v/v))] and close the jar securely to prevent spillage.

(3) Blend on high setting for 2 minutes.

(4) Filter the extract through a Whatman #1 filter into a clean container that is labeled with a sample ID number. Cap the container to prevent the evaporation of solvents.

(5) Dilute 1 part of the filtered extract with 1 part of distilled/deionized water. (e.g., mix 1 mL filtered extract with 1 mL water). This is the **diluted filtrate extract** and is ready for testing.

(6) Use 50 µL of the diluted filtrate extract per well for testing.

TEST PROCEDURES

a. <u>Analysis Procedure</u>.

(1) Allow reagents and antibody wells to reach room temperature (68 - 77º F) prior to running the test.

(2) Only 1 control standard (zero standard) is included in the test kit. The standard curve (B/Bo) is provided with the certificate of the test kit.

(3) Insert a sufficient number of wells into the microwell holder for control standard and samples to be tested. (For example: to test 15 samples use 16 wells - 1 for the control standard and 15 for the test samples).

NOTE: Do not run more than 2 strips (15 samples) per run.

(4) Using a new pipette tip for the zero (0) control standard and each test sample, pipette 50µL of standard and prepared sample(s) to separate wells.

(5) Add 50 µL of enzyme conjugate (red capped bottle) into each well using a repeating pipettor with a 2.5 mL tip on setting 1.

(6) Add 50 µL of Anti-aflatoxin antibody (black capped bottle) into each well using a repeating pipettor with a 2.5 mL tip on setting 1.

(7) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.

(8) Incubate for **10 minutes** (± 1 minute) at room temperature. Cover wells to protect them from light.

(9) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(10) Using a wash bottle, fill each well with washing buffer solution. Empty the wells and tap a few times on a paper towel. Repeat this step 2 more times (total of 3 washes). Ensure wells are completely free of liquid after third wash prior to moving to the next step.

(11) Add 100 µL of substrate/chromogen (brown cap brown plastic bottle) to each well using a repeating pipettor with a 5 mL tip on setting 1.

(12) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.

(13) Incubate for **5 minutes** (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(14) Add 100 µL of stop solution (yellow cap-brown glass bottle) to each well using a repeating pipettor with a 5 mL tip on setting 1.

(15) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for **10-15 seconds** without spilling reagents.

(16) Measure absorbance at 450 nm using the Awareness Technology Stat-Fax Model 303 PLUS (results must be read within 10 minutes).

b. <u>Reading the Results</u>

(1) Stat-Fax Model 303 PLUS Microwell Reader.

(a) Press Menu, the prompt should read: “Select Test” Press 1, then ENTER.

(b) The concentrations and B/BO% should now be printing.

(2) Display will read: “New B/BO Number Y/N (Yes/No)”. Press "N" if the B/BO matches the QC sheet in the test kit in use. Press “Y” if the B/BO on the printout does not match the QC sheet in the kit.

(3) If “Y” was pressed for new B/BO, it will now display: Cal 2 B/BO%=____simply insert the B/BO number from the QC sheet for standard 2 and press ENTER.

(4) When completed the reader will print “Test is Updated”.

Note: Please verify new B/BO number entered on the printout match test kit QC sheet.

(5) If “N” was pressed for new B/BO, or you just finished updating the B/BO , it will now display: “Set carrier to 1; press Enter”

(6) Place the wells in the far right column of the carrier with the zero (0) standard at the top.

(7) Align carrier to the far left for column 1. Then press ENTER.

(8) The reader is now reading the first eight wells. Once complete the display will read: "Plot Curve Y/N. Select N.

(9) Display will now read: "Accept Curve Y/N".

If you are only running one strip, the test is now complete (press the clear button twice). If you have an additional strip to run, select yes. Move the carrier to the right so that the wells are aligned with notch in the center. Now press ENTER.

(10) The reader is now reading the second set of eight wells.

(11) Once the last strip is read, press the clear button twice.

(12) Test is now complete.

SUPPLEMENTAL ANALYSIS

1. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 100 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 100 ppb, the sample extract must be diluted so that a value between 5 and 100 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

2. Example.

If the original analysis reported the aflatoxin value at greater than 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

a. Prepare a 35% methanol dilution solvent by adding equal portions of distilled or deionized water and 70/30 methanol/water extraction solvent. Example: 10 mL of water plus 10 mL of 70/30 methanol mixture will provide 20 mL of 35% methanol in water.

b. Dilute 200 µL (0.2 mL) of the diluted extract (obtained from the original extract as applicable) with 1.8 mL of the dilution solvent mixture from step "a" above. The total volume is 2 mL. This is a 1:9 dilution, Dilution Factor (DF) is 10. Proceed to sample test analysis section.

c. Multiply the analytical results obtained by ten (**10**) to obtain the actual

aflatoxin concentration. For example, if 25 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 250 ppb.

A final result of less than 53 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 100 ppb.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

b. Precautions

(1) Do not interchange individual reagents between kits of different lot numbers.

(2) Do not use the test kits beyond the noted expiration date.

(3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (48 well kit).

(1) 1 Microtiter plate with 48 wells (6 strips with removable wells) each coated with capture antibodies.

(2) 1 Afla standard solution of 1.3 mL 0 ppm (zero standard).

(3) 1 red-capped bottle of 3 mL peroxidase conjugated aflatoxin solution.

(4) 1 black-capped bottle of 3 mL anti- aflatoxin antibody.

(5) 1 brown-capped brown plastic bottle of 6mL substrate/chromogen, stained red.

(6) 1 yellow-capped brown glass bottle of 6mL stop solution.

(7) 1 packet of washing buffer (salt).

b. <u>Materials Required but not Provided.</u>

(1) Awareness Technology Inc. Stat-Fax Model 303 PLUS with 450-nm filter.

(2) 50 µL, 100 µL, and 1000 µL pipettor and pipette tips.

(3) Graduated cylinders (plastic or glass): 250 mL and 1 L.

(4) Blender, blender jars, blades, and lids with gaskets

(5) Filter funnel.

(6) Whatman #1 filter paper or equivalent.

(7) Balance.

(8) Repeating pipettor and 2.5/5.0mL tips.

(9) Paper towels, Kay dry paper or equivalent absorbent material.

(10) Waste receptacle.

(11) Timer: 3 channel minimum.

(12) Waterproof marker, Sharpie or equivalent.

(13) Wash bottle.

(14) Deionized or distilled water.

(15) Methanol.

Revision History:

Revision 0 04/21/2016